DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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NANOSPHERE, INC.
NOAH LERMER, Ph.D
DIRECTOR, REGULATORY AFFAIRS
4088 COMMECIAL AVENUE
NORTHBROOK IL 60062

October 10, 2014

Re: K142033
Trade/Device Name: Verigene® Enteric Pathogens Nucleic Acid Test (EP)
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal microorganism multiplex nucleic acid-based assay
Regulatory Class: II
Product Code: PCH, PCI, OOI
Dated: July 24, 2014
Received: July 25, 2014

Dear Dr. Lermer:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K142033

Device Name
Verigene Enteric Pathogens Nucleic Acid Test (EP)

Indications for Use *(Describe)*
The Verigene Enteric Pathogens Nucleic Acid Test (EP) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and viruses:

- Campylobacter Group (composed of C. coli, C. jejuni, and C. lari)
- Salmonella species
- Shigella species (including S. dysenteriae, S. boydii, S. sonnei, and S. flexneri)
- Vibrio Group (composed of V. cholerae and V. parahaemolyticus)
- Yersinia enterocolitica
- Norovirus GI/GII
- Rotavirus A

In addition, EP detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing E. coli (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2.
EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. EP also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.
Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for Yersinia enterocolitica, Vibrio Group and Shigella species were primarily established with contrived specimens.
Concomitant culture is necessary for organism recovery and further typing of bacterial agents.
EP results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative EP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

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510(K) Summary

510(k) Number:

K142033: Verigene® Enteric Pathogens Nucleic Acid Test (EP)

Summary Preparation Date:

September 25, 2014

Submitted by:

Nanosphere, Inc.
4088 Commercial Avenue
Northbrook, IL 60062
Phone: 847-400-9000
Fax: 847-400-9176

Contact:
Noah Lermer
Director, Regulatory Affairs

Proprietary Names:

For the instrument:

Verigene® System

For the assay:

Verigene® Enteric Pathogens Nucleic Acid Test (EP)

Verigene® EP

Common Names:

For the instrument:

Bench-top molecular diagnostics workstation

For the assay:

Enteric Pathogens Nucleic Acid Test
Enteric Pathogens identification and differentiation system
Enteric assay
Enteric test

Regulatory Information:

Regulation section:

866. 3990 - Gastrointestinal microorganism multiplex nucleic acid-based assay

Classification:

Class II

Panel:

Microbiology (83)

Product Code(s):

PCH Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System
PCI Gastrointestinal Bacterial Panel Multiplex Nucleic Acid-based Assay System
OOI Real Time Nucleic Acid Amplification System

Other codes used by predicate devices:

NSU Instrumentation for clinical multiplex test systems
JJH Clinical Sample Concentrator

Predicate Devices:

xTAG Gastrointestinal Pathogen Panel (GPP) (K121894/K121454) (Luminex Molecular Diagnostics, Inc.)

Indications for Use:

The Verigene Enteric Pathogens Nucleic Acid Test (**EP**) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and viruses:

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- *Yersinia enterocolitica*
- Norovirus GI/GII
- Rotavirus A

In addition, **EP** detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2.

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Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

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Technological Characteristics:

The Verigene Enteric Pathogens Nucleic Acid Test (EP) is a molecular assay that relies on detection of specific nucleic acid targets in a microarray format. For each of the bacterial or viral nucleic acid sequences detected by EP, unique Capture and Mediator oligonucleotides are used, with gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides are covalently bound to the microarray substrate and hybridize to a specific portion of the nucleic acid targets. The Mediator oligonucleotides have a region that binds to a different portion of the same nucleic acid targets and also have a sequence that allows binding of a gold nanoparticle probe. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency and provide accurate detection of target capture.

The EP test is performed on the Verigene System, a "sample-to-result," fully automated, bench-top molecular diagnostics workstation. The System enables automated nucleic acid extraction from unformed stool specimens (liquid or soft) preserved in Cary-Blair media and detection of analyte-specific target nucleic acids. The Verigene System consists of two components: the Verigene Reader and the Verigene Processor *SP*.

The Reader is the Verigene System's user interface and serves as the central control unit for all aspects of test processing, automated imaging, and result generation using a touch-screen control panel and a barcode scanner. The Verigene Processor *SP* executes the test procedure, automating the steps of (1) Sample Preparation and Target Amplification – cell lysis and magnetic bead-based bacterial and viral nucleic acid isolation and amplification, and (2) Hybridization– detection and identification of analyte-specific nucleic acid in a microarray format by using gold nanoparticle probe-based technology. Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of the trays and finally loads the specimen into the Test Cartridge for hybridization. Single-use disposable test consumables and a self-contained Verigene Test Cartridge are used for each sample tested with the EP assay.

To obtain the test results after test processing is complete, the user removes the Test Cartridge from the Processor *SP*, and inserts the substrate holder into the Reader for analysis. Light scatter from the capture spots is imaged by the Reader and intensities from the microarray spots are used to make a determination regarding the presence (Detected) or absence (Not Detected) of a targeted nucleic acid sequence/analyte. This determination is made by means of software-based decision algorithm resident in the Reader.

Performance Data - Analytical Testing

Note: Please refer to K140083 for information on EP test analytical performance for bacterial and Shiga toxin gene virulence marker targets.

Analytical Sensitivity / Limit of Detection (LoD)

Analytical sensitivity (LoD) of the EP test for Norovirus and Rotavirus was established by testing four (4) representative virus strains. The LoD is defined as the concentration at which the test produces a positive result at least 95% of the time. Serial dilutions of the strains were tested in replicates of four and the putative LoD was confirmed with 20 replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, a further 20 replicates were performed at the next lower concentration until <95% was achieved. The LoDs for the 4 strains tested, and the corresponding LoD ranges for the EP test reportable target, are shown in the table below. Overall, the LoD ranged from 4.12×10^5 – 1.67×10^6 copies/mL of stool for Norovirus and 3.70×10^2 – 1.11×10^3 $TCID_{50}$/mL of stool for Rotavirus.

Organism	***LoD (/mL Stool)***
Norovirus – GI (CDC 2142)	4.12×10^5 copies
Norovirus – GII (D17219)	1.67×10^6 copies
Rotavirus – DS1 (VR-2550)	1.11×10^3 $TCID_{50}$
Rotavirus – W161 (VR-2551)	3.70×10^2 $TCID_{50}$

Analytical Reactivity (Inclusivity)

Analytical Reactivity of the EP test for Norovirus and Rotavirus was demonstrated with a comprehensive panel of 41 clinically relevant viral strains representing temporal, geographical, and phylogenic diversity for each claimed target (see table below). All of the Rotavirus and most of the Norovirus strains tested generated the expected result when tested in triplicate at a concentration of 3x LoD. For some of the Norovirus GII strains, slightly higher concentrations were required to demonstrate inclusivity (10x-50x LoD).

Norovirus strains GII.9, GII.14, and GIV.1 and Rotavirus A strains G4, G5, G10, G11, and G15 are predicted to be detected based on *in silico* analysis. Norovirus GII.11 is not expected to be detected by the EP test. Additionally, based on *in silico* analysis, rare Norovirus genotypes GII.6 and GII.13 are predicted to either be not detected by EP or to be detected with reduced sensitivity. Inclusivity to Norovirus strains GII.8 and Rotavirus G7, G21, and G24 could not be evaluated due to a lack of available sequences.

Reportable Target	***Number of Organisms/Strains Tested***	***Genogroups (No. Of Strains)***
Norovirus	29	GI (13), GII (16)
Rotavirus	12	Group A (12)

Analytical Specificity (Cross-reactivity)

One-hundred and fifty-eight (158) organisms, consisting of 134 bacterial organisms, 18 viruses, four (4) parasites, one (1) fungal organism, and one (1) human cell line were tested with the EP test to determine analytical specificity (see table below). Eight (8) organisms, including Astrovirus and Sapovirus (2 strains), *Campylobacter hominis*, and four (4) parasites were evaluated by using genomic DNA/RNA. In addition, the cross-reactivity of 15 species of *Vibrio* not associated with human infection, four (4) non-pathogenic strains of *Escherichia coli*, *Yersinia pestis*, *Clostridium botulinum*, Rotavirus (Genogroups B, C, D, NADRV), Adenovirus (Species G), and Norovirus (Genotype GIV.2 and Genogroups GIII, GV) were evaluated by *in silico* analysis. Finally, to rule out cross-reactivity between the analytes detected by the EP test, nine representative in-panel organisms were tested at elevated concentrations.

All of the tests yielded the expected "Not Detected" results, indicating that there was no cross-reactivity with the EP test Norovirus and Rotavirus probes. Additionally, with the exception of porcine strains of Rotavirus C, all of the organisms evaluated for exclusivity through *in silico* analysis are predicted to not be detected by the EP test.

Exclusivity Testing: Bacterial Non-Test Panel Members			
Genus	**Species**	**Genus**	**Species**
Abiotrophia	*defectiva*	*Escherichia*	*coli (3 strains)*
Acinetobacter	*baumannii*		*coli* (EAEC)
	lwoffii		*coli* (EPEC) *(2 strains)*
Arcobacter	*butzleri*		*coli* (ETEC) *(2 strains)*
	cryaerophilus		*fergusonii*
Aeromonas	*allosaccharophila*		*hermannii*
	bestiarum	*Fusobacterium*	*varium*
	caviae	*Helicobacter*	*hepaticus*
	encheleia		*pylori (4 strains)*
	enteropelogenes	*Klebsiella*	*oxytoca*
	eucrenophila		*pneumoniae*
	hydrophilia	*Lactobacillus*	*acidophilus*
	jandaei		*reuteri*
	*salmonicida**		*rhamnosus*
	veronii	*Lactococcus*	*lactis*
Alcaligenes	*faecalis*	*Leminorella*	*grimontii*
Bacillus	*cereus*	*Listeria*	*grayi*
Bacteroides	*caccae*		*monocytogenes*
	fragilis	*Morganella*	*morganii*
	merdae	*Peptostreptococcus*	*anaerobius*
	stercoris	*Plesiomonas*	*shigelloides*
Cedecea	*davisae*	*Porphyromonas*	*asaccharolytica*
Citrobacter	*amalonaticus*	*Prevotella*	*melaninogenica*
	freundii	*Proteus*	*mirabilis*
	sedlakii		*vulgaris*
Clostridium	*bifermentans*		*penneri*
	bolteae	*Providencia*	*stuartii*
	butyricum		*alcalifaciens*
	difficile (2 strains)		*rettgeri*
	difficile, non-tox	*Pseudomonas*	*aeruginosa (2 strains)*
	haemolyticum		*fluorescens*
	methylpentosum		*putida*
	nexile	*Ruminococcus*	*bromii*
	noyvi	*Serratia*	*liquefaciens*
	orbiscindens		*marcescens*
	perfringens	*Staphylococcus*	*aureus*
	scindens		*epidermidis*
	septicum	*Streptococcus*	*agalactiae, O90R*
	sordellii		*dysgalactiae*
	spiroforme		*mutans*
	sporogenes	***Parasites***	
Collinsella	*aerofaciens*	*Blastocystis*	*hominis*
Desulfovibrio	*piger*	*Cryptosporidium*	*parvum*
Edwardsiella	*tarda*	*Entamoeba*	*histolytica*
Enterobacter	*aerogenes*	*Giardia*	*lamblia*
	cloacae	***Human Cell Line***	
Enterococcus	*faecalis*	Colon epithelial cells	
	faecium	***Fungal Strain***	
		Candida albicans	

** Sub-species masoucida and sub-species salmonicida (2 strains)*

Campylobacter, Vibrio, and Yersinia Species Not Detected by EP	
Genus	**Species**
Campylobacter	*concisus*
	curvus
	fetus
	gracilis
	hominis
	hyointestinalis
	insulaenigrae
	lanienae
	mucosalis
	rectus
	showae
	sputorum
	upsaliensis
Vibrio	*alginolyticus*
	campbellii
	cincinnatiensis
	fluvialis
	furnissii
	harveyi
	metschnikovii
	mimicus
	tubiashii
	vulnificus (3 strains)
Yersinia	*aldovae*
	aleksiciae
	bercovieri
	frederiksenii
	intermedia
	kristensenii
	mollaretii
	pseudotuberculosis
	ruckeri
	rohdei
Viruses	
Name	**Serovar / Group**
Adenovirus	Type 1/Group C
	Type 2/Group C
	Type 3/Group B1
	Type 4/Group E
	Type 5/Group C
	Type 14/Group B2
	Type 26/Group D
	Type 31/Group A
	Type 37/Group D
	Type 40/Group F
	Human 4
Astrovirus	-
Coxsackievirus B4	-
Cytomegalovirus	-
Echovirus 11	-
Enterovirus 68	-
Sapovirus (2 strains)	-

Microbial Interference

Potential interference of 14 microorganisms was evaluated with representative strains of Norovirus and Rotavirus. These microorganisms, *Bacteroides fragilis, Prevotella oralis, Prevotella melaninogenica, Bifidobacterium bifidum, Clostridium perfringens, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Lactobacillus acidophilus, Staphylococcus aureus, Blastocystis hominis, Entamoeba histolytica,* and *Candida albicans,* represent the most prevalent organisms in the human colon and therefore are the most likely to be encountered in stool specimens tested with the EP test. The microorganisms were tested at a concentration of 10^7 CFU/mL, with the exception of the parasites *Blastocystis hominis* and *Entamoeba histolytica*, which were tested at 9×10^6 cells/mL and 7×10^5 cells/mL, respectively. No interference was observed with the EP test for any of the samples tested.

Interference (Exogenous Substances)

A comprehensive interfering substances study was performed to assess the potential inhibitory effect of endogenous and exogenous substances that can commonly be found in clinical stool specimens. Representative strains of Norovirus and Rotavirus were individually challenged with 22 potentially interfering substances (shown below) at high, medically-relevant concentrations. None of the 22 substances tested showed any inhibitory effect on the EP test detection of the target viruses.

- Intralipid (Triglyceride - Fecal Fat)
- Cholesterol (Cholesterol - Fecal Fat)
- Whole Blood
- Mucus (Nasopharyngeal swab sample in UTM)
- Nystatin Suspension
- Preparation H® Anti-itch Hydrocortisone 1%
- Desitin Maximum Strength Original Paste
- Preparation H® Hemorrhoidal Ointment
- Options Conceptrol® Vaginal Contraceptive Gel
- Wet Ones® Antibacterial Hand Wipes
- K-Y® Personal Lubricant Jelly
- Vaseline Original 100% Pure Petroleum Jelly
- Tums Antacid with Calcium Extra Strength 750
- Gaviscon Extra Strength Liquid Antacid
- Mesalazine
- Imodium® AD Anti-Diarrheal
- Pepto-Bismol Max Strength
- Metronidazole Topical Cream (0.75%)
- Naproxen Sodium
- Mucin from bovine submaxillary glands, Type I-S
- Barium Sulfate
- Amoxicillin (Antibiotic)

Competitive Inhibition

The potential for competitive inhibition of the EP test for the viral targets was evaluated by testing 26 unique binary combinations of Norovirus and Rotavirus with the EP test panel organisms. Simulated samples were prepared in Negative Stool Matrix (NSM), with one panel organism at a Low Positive titer (3x LoD) and a second organism at a High Positive titer (10-100-fold higher than the organism's LoD). The EP test correctly detected all organisms present in the co-infection combinations tested in replicates of three (3) with one exception: the *"Low Titer Rotavirus and High Titer Y. enterocolitica*" sample the EP test correctly identified *Y. enterocolitica* in all cases, but *Rotavirus* was detected only in two (2) of the three (3) replicates.

An additional 6 replicates were tested and the expected result was obtained for both analytes in all replicates.

Cut-off Verification

Target mean intensity values were examined from the testing of sixteen (16) bacterial strains and four (4) viral strains used to establish the Limit of Detection of the EP test. In addition, the cut-off dataset included the test results of two (2) negative samples. With replicates of 20 for each sample and fourteen (14) unique target spot groups evaluated per test, a total of 6160 data points (1320 expected positive) were assessed to verify the assay cut-off.

Carryover / Cross-contamination

The potential for carryover and cross-contamination of the EP test on the Verigene system was assessed by alternately testing 3 representative viral samples with negative stool samples across multiple Verigene Processor *SP*s. No carryover or cross-contamination was observed.

Precision

The EP test precision study for the viral targets was conducted in-house by Nanosphere as part of the full 20-sample precision study. The full panel was composed of three (3) different viral strains and six (6) different EP panel bacterial strains, each at two (2) concentrations, as well as two (2) negative samples (Negative Stool Matrix and *Clostridium difficile*). In the study, each sample was tested daily in duplicate by two (2) operators for four (4) non-consecutive days for a total of sixteen (16) tests per sample (2 operators / day x 2 replicates / operator x 4 days).

Results for the viral strains are summarized below.

Sample ID	***Expected EP Call***	***Concentration***	***Agreement with Expected Result (95 % CI)***[a]
Rotavirus	Rotavirus	Moderate	16/16 100% (79.4%-100%)
		Low	16/16 100% (79.4%-100%)
Norovirus GI	Norovirus	Moderate	16/16 100% (79.4%-100%)
		Low	16/16 100% (79.4%-100%)
Norovirus GII	Norovirus	Moderate	16/16 100% (79.4%-100%)
		Low	16/16 100% (79.4%-100%)

[a] 95% Two-sided Exact Binomial Confidence Interval calculation using the exact Clopper-Pearson method.

Performance Data - Clinical Testing

Reproducibility

The inter-laboratory reproducibility of the EP test was determined by conducting a reproducibility study at three external sites. Three (3) different viral strains at two (2) concentrations were tested as part of the full 20-sample EP test reproducibility study, which also included six (6) different EP panel bacterial strains at two concentrations, as well as two (2) negative samples (Negative Stool Matrix and *Clostridium difficile*). The samples were tested daily in triplicate by two (2) operators for five (5) non-consecutive days at three (3) sites for a total of ninety (90) tests per sample. The results of the Reproducibility Study for viral samples are provided in the table below.

Sample	***Expected Call***	***Concentration***	***Agreement with Expected Result (95 % CI)***			
			Site 1	*Site 2*	*Site 3*	*Total*
Rotavirus	Rotavirus Detected	*Moderate*	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)	90/90 100% (96.0-100)
		Low	30/30 100% (88.4-100)	27/30 90.0% (73.5-97.9)	30/30 100% (88.4-100)	87/90 96.7% (90.6-99.3)
Norovirus GI	Norovirus Detected	*Moderate*	30/30 100% (88.4-100)	30/30 100% (88.4-100)	28/30 93.3% (77.9-99.2)	86/88 97.7% (92.0-99.7)
		Low	30/30 100% (88.4-100)	28/30 93.3% (77.9-99.2)	28/30 93.3% (77.9-99.2)	86/90 95.6% (89.0-98.8)
Norovirus GII	Norovirus Detected	*Moderate*	30/30 100% (88.4-100)	30/30 100% (88.4-100)	29/30 96.7% (82.8-99.9)	86/87 98.9% (93.8-100)
		Low	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)	89/89 100% (95.9-100)

Clinical Study - Method Comparison

The performance characteristics of the EP test for Norovirus and Rotavirus were determined by comparing the Verigene EP test results to PCR-based viral reference methods in a multi-site prospective investigation study at eight (8) U.S. institutions. A total of 1940 valid specimens were evaluated in the study which included 1294 prospectively-collected fresh specimens, 34 prospectively-collected frozen specimens, 203 selected samples, and 409 simulated specimens.

The following table provides a summary of demographic information for 1313 of the 1328 prospectively-collected specimens in the valid dataset.

Age Range	***No. of Specimens***	***Percentage***
0-1	63	4.8%
>1-5	49	3.7%
>5-12	85	6.5%
>12-21	146	11.1%
>21-65	636	48.4%
>65	334	25.4%
Total	**1313**	**100%**

The viral comparator methods were a composite of a real-time RT-PCR assay and conventional PCR assays with confirmatory bi-directional sequencing. The PCR assays were designed to amplify different gene regions than those targeted by the EP test. The tables below provide a summary of the clinical performance of the EP test (n=1940), compared to the reference/comparator methods and stratified by specimen type, for the detection of Norovirus and Rotavirus, as well as the five (5) bacterial targets and the Stx1 and Stx2 targets.

	Specimen Type			***n***	***% Agreement (95% CI)***	
					Positive	***Negative***
Norovirus GI/GII	Clinical Specimens	Prospectively Collected	Fresh	1294	94.9% 37/39 (82.7-99.4)	99.6% 1250/1255 (99.0-99.9)
			Frozen	34	0% 0/1 (0.0-97.5)	100% 33/33 (89.7-100)
		Selected		203	100% 18/18 (81.5-100)	99.5% 184/185 (97.0-100)
	Simulated			409	-	100% 409/409 (99.1-100)

	Specimen Type			***n***	***% Agreement (95% CI)***	
					Positive	***Negative***
Rotavirus A	Clinical Specimens	Prospectively Collected	Fresh	1294	66.7% 2/3 (9.4-99.2)	99.9% 1290/1291 (99.6-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	98.0% 50/51 (89.6-100)	100% 152/152 (97.6-100)
	Simulated			409	-	100% 409-409 (99.1-100)

	Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative
Campylobacter spp.	Clinical Specimens	Prospectively Collected	Fresh	1294	90.9% 20/22 (79.8-98.9)	98.7% 1255/1272 (97.9-99.2)
			Frozen	34	100% 2/2 (15.8-100)	100% 32/32 (89.1-100)
		Selected		203	97.5% 39/40 (86.8-99.9)	99.4% 162/163 (96.6-100)
	Simulated			409	98.5% 67/68 (92.1-100)	100% 341/341 (98.9-100)
Salmonella spp.	Clinical Specimens	Prospectively Collected	Fresh	1294	86.4% 19/22 (65.1-97.1)	99.4% 1265/1272 (98.9-99.8)
			Frozen	34	100% 1/1 (2.5-100)	97.0% 32/33 (84.2-99.9)
		Selected		203	98.3% 58/59 (90.9-100)	99.3% 143/144 ((96.2-100)
	Simulated			409	100% 67/67 (94.6-100)	100% 342/342 (98.9-100)
Shigella spp.	Clinical Specimens	Prospectively Collected	Fresh	1294	66.7% 2/3 (9.4-99.2)	98.8% 1275/1291 (98.0-99.3)
			Frozen	34	-	97.1% 33/34 (84.7-99.9)
		Selected		203	100% 8/8 (63.1-100)	99.5% 194/195 (97.2-100)
	Simulated			409	100% 50/50 (92.9-100)	100% 359/359 (99.0-100)
Vibrio spp.	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 1/1 (2.5-100)	100% 1293/1293 (99.7-100)
			Frozen	34	100% 1/1 (2.5-100)	100% 33/33 (89.4-100)
		Selected		203	100% 1/1 (2.5-100)	100% 202/202 (98.2-100)
	Simulated			409	91.1% 51/56 (80.4-97.0)	99.7% 352/353 (98.4-100)
Y. enterocolitica	Clinical Specimens	Prospectively Collected	Fresh	1294	-	100% 1294/1294 (99.7-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 1/1 (2.5-100)	100% 202/202 (98.2-100)
	Simulated			409	100% 59/59 (93.9-100)	100% 350/350 (99.0-100)

	Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative
Stx1	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 4/4 (39.8-100)	99.8% 1287/1290 (99.3-100
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 9/9 (66.4-100)	99.5% 193/194 (97.2-100)
	Simulated			409	100% 50/50 (92.9-100)	99.2% 356/359 (97.6-99.8)

	Specimen Type			n	% Agreement (95% CI)	
					Positive	Negative
Stx2	Clinical Specimens	Prospectively Collected	Fresh	1294	100% 6/6 (54.1-100)	99.8% 1286/1288 (99.4-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		203	100% 10/10 (69.2-100)	100% 193/193 (98.1-100)
	Simulated			409	96.6% 57/59 (88.3-99.6)	99.4% 348/350 (98.0-99.9)

Substantial Equivalence

The Verigene Enteric Pathogen Nucleic Acid Test (EP test) for Norovirus GI/GII and Rotavirus A targets has been shown to be substantially equivalent to the xTAG Gastrointestinal Pathogen Panel (GPP). The EP test has similar intended use and indications, technological characteristics, and performance characteristics. The minor differences between the EP test and its predicate device raise no new issues of safety or effectiveness. Performance data demonstrate that the EP test is as safe and effective as the predicate device. Thus, the EP test is substantially equivalent to the predicate device.

Similarities		
Element	**New Device:** **Enteric Pathogens Nucleic Acid Test (EP)** **K142033**	**Predicate:** **xTAG Gastrointestinal Pathogen Panel (GPP)** **K121894**
Intended Use	The Verigene Enteric Pathogens Nucleic Acid Test (**EP**) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria, viruses and genetic virulence markers from liquid or soft stool preserved in Cary-Blair medium, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria and viruses: • *Campylobacter* Group (composed of *C. coli*, *C. jejuni*, and *C. lari*) • *Salmonella* species • *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*) • *Vibrio* Group (composed of *V. cholerae* and *V. parahaemolyticus*) • *Yersinia enterocolitica* • Norovirus GI/GII • Rotavirus A In addition, **EP** detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2. **EP** is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. **EP** also aids in the detection and identification of acute	The xTAG Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG GPP: • *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only) • *Clostridium difficile (C. difficile) toxin A/B* • *Cryptosporidium (C. parvum and C. hominis only)* • *Escherichia coli (E. coli) O157* • *Enterotoxigenic Escherichia coli (ETEC) LT/ST* • *Giardia (G. lamblia only - also known as G. intestinalis and G. duodenalis)* • *Norovirus GI/GII* • *Rotavirus A* • *Salmonella* • *Shiga-like Toxin producing E. coli (STEC) stx 1/stx 2* • *Shigella (S. boydii, S. sonnei, S. flexneri and S. dysenteriae)* The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the

Similarities		
Element	**New Device: Enteric Pathogens Nucleic Acid Test (EP) K142033**	**Predicate: xTAG Gastrointestinal Pathogen Panel (GPP) K121894**
	gastroenteritis in the context of outbreaks. Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. **EP** results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative **EP** results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.	detection and identification of acute gastroenteritis in the context of outbreaks. xTAG GPP positive results are presumptive and must be confirmed by FDA cleared tests or other acceptable reference methods. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out coinfection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG Gastrointestinal Pathogen Panel results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. xTAG GPP is not intended to monitor or guide treatment for *C. difficile* infections. The xTAG GPP is indicated for use with the Luminex MAGPIX instrument.
Specimen Type	Human Stool sample in Cary-Blair Media	Same
DNA Amplification	PCR	Same
Organisms/NA Targets Detected	*Campylobacter* Group (*C. coli, C. jejuni,* and *C. lari)* *Salmonella* species *Shigella* species (*S. dysenteriae, S. boydii, S. sonnei,* and *S. flexneri)* *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*) *Yersinia enterocolitica* Norovirus GI/GII Rotavirus A Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers	Same with additional analytes (excluding *Vibrio* Group and *Yersinia enterocolitica*).

Differences		
Element	**New Device: Enteric Pathogens Nucleic Acid Test (EP) K142033**	**Predicate: xTAG Gastrointestinal Pathogen Panel (GPP) K121894**
Time to Result	~ 2 hours	5 hours
Sample prep	On-board, automated NA extraction and amplification	Off-line NA Extraction and amplification
Detection Method	Gold/Silver nanoparticle probe detection of microbial-specific DNA on complementary oligo- microarray	Specific microbial target or control bead populations coupled to sequences from Universal Array streptavidin, R-phycoerythrin conjugate
Optical Detection	Light scatter	Multi-color fluorescence